

We claim:

- Sub B²
1. An isolated nucleic acid molecule that prolongs the expression of cold shock inducible genes under conditions that elicit the cold shock response in a bacterium.
 2. The nucleic acid molecule of Claim 1, wherein said molecule comprises a 5'-UTR of a cold shock inducible gene or a substantially homologous sequence thereof.
 3. The nucleic acid molecule of Claim 2, wherein said 5'-UTR is a 5'-UTR of a cold-shock inducible gene selected from the group consisting of *cspA*, *cspB* and *csdA*.
 4. The isolated nucleic acid molecule of Claim 2, wherein said 5'-UTR comprises a cold box or a substantially homologous sequence thereof.
 - Sub A⁶ 5. The nucleic acid molecule of Claim 3, wherein said 5'-UTR comprises nucleotides +1 to +11 of the *cspA* 5'-UTR or a substantially homologous sequence thereof.
 - Sub B² 6. The nucleic acid molecule of Claim 1, wherein said cold shock inducible gene interacts with CspA protein.
 7. An isolated nucleic acid molecule that represses the expression of cold shock inducible genes under physiological conditions.
 8. The isolated nucleic acid molecule of Claim 7, comprising at least a portion of the 5'-UTR of a cold shock inducible gene.
 9. The isolated nucleic acid molecule of Claim 8, wherein said cold-shock inducible gene is selected from the group consisting of *cspA*, *cspB*, and *csdA*.
 - Sub A⁷ 10. The nucleic acid molecule of Claim 8 wherein said cold-shock inducible gene comprises nucleotides +56 to +117 of *cspA* or nucleotides having substantial homology to nucleotides +56 to +117 of *cspA*.
 - Sub B³ 11. A non-coding nucleic acid molecule that enhances the translation of cold shock inducible genes under conditions that elicit the cold shock response of a bacterium.

sub B3
12. The nucleic acid molecule of Claim 11 comprising at least a portion of the 5'-UTR of a cold shock inducible gene.

13. The nucleic acid molecule of Claim 12 wherein said cold shock inducible gene is selected from the group consisting of *cspA*, *cspB*, and *csdA*.

5 sub AD
14. The nucleic acid molecule of Claim 13, comprising nucleotides +123 to +135 of *cspA* or nucleotides having substantial homology to +123 to +135 of *cspA*.

sub B3
15. The nucleic acid molecule of Claim 14 comprising having a sequence selected from the group consisting of SEQ ID NO:48, SEQ ID NO:49, and SEQ ID NO:50.

10
16. A nucleic acid vector comprising a downstream box and a nucleic acid molecule that enhances translation of cold shock inducible genes under conditions that elicit the cold shock response in a bacterium.

17. The construct of Claim 16 further comprising a Shine-Dalgarno sequence.

15
18. A nucleic acid vector wherein at least a portion of said vector comprises a cold box, a translational enhancer, and a downstream box; said vector directing prolonged expression and enhancing translation of a gene under conditions that elicit a cold shock response in a bacterium.

20
19. A nucleic acid vector that directs the prolonged expression and enhances the translation of a gene under conditions of physiological stress that elicit the cold shock response of a bacterium, and represses the expression of the gene under physiological conditions comprising a cold box, at least a portion of the 5'-UTR of a cold-shock inducible gene that represses the expression of cold-shock inducible genes, at least a portion of the 5'-UTR of a cold-shock inducible gene that enhances translation of cold-shock genes, and a downstream box.

20. The vector of claim 16 wherein said the gene is a cold shock inducible gene.

25
21. The vector of Claim 18, wherein said gene is a cold-shock inducible gene.

sub
B³

5

22. The vector of Claim 19, wherein said gene is a cold-shock inducible gene.
23. The vector of Claim 16 wherein said gene is a heterologous gene.
24. The vector of Claim 18 wherein said gene is a heterologous gene.
25. The vector of Claim 19, wherein said gene is a heterologous gene.
26. The vector of Claim 16, further comprising a promoter and at least one restriction site downstream of said 5' UTR and said downstream box for inserting an additional DNA fragment.
27. The vector of Claim 18, further comprising a promoter and at least one restriction site downstream of said 5' UTR and said downstream box for inserting an additional DNA fragment.
28. The vector of Claim 19, further comprising a promoter and at least one restriction site downstream of said 5' UTR and said downstream box for inserting an additional DNA fragment.
29. The vector of claim 26, wherein said additional DNA fragment encodes a cold shock inducible gene.
30. The vector of 27, wherein said additional DNA fragment encodes a cold shock inducible gene.
31. The vector of Claim 28, wherein said additional DNA fragment encodes a cold shock inducible gene.
32. The vector of Claim 26, wherein said additional DNA fragment encodes a heterologous gene.
33. The vector of Claim 27, wherein said additional DNA fragment encodes a heterologous gene.
34. The vector of Claim 28, wherein said additional DNA fragment encodes a heterologous gene.

10

15

20

25

35. A transformed bacteria containing the vector of Claim 16.
36. A transformed bacteria containing the vector of Claim 18.
37. A transformed bacteria containing the vector of Claim 19.
38. A method for overexpressing a gene comprising transforming bacteria with a nucleic acid vector of Claim 16 and subjecting said bacteria to conditions that elicit a cold shock response.
39. A method for overexpressing a gene comprising transforming bacteria with a nucleic acid vector of Claim 18 and subjecting said bacteria to conditions that elicit a cold shock response.
40. A method for overexpressing a gene comprising transforming bacteria with a nucleic acid vector of Claim 19 and subjecting said bacteria to conditions that elicit a cold shock response.
41. The method of Claim 37, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.
42. The method of Claim 38, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.
43. The method of Claim 39, wherein said overexpression causes a reduction in the expression of at least one endogenous protein.
44. The method of Claim 37, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.
45. The method of Claim 38, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

46. The method of Claim 39, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

47. The method of Claim 43, wherein said temperature is about 10-15°C.

5

48. The method of Claim 44, wherein said temperature is about 10-15°C.

49. The method of Claim 45, wherein said temperature is about 10-15°C.

Sub B6
10
50. A vector capable of expressing a heterologous gene in a bacterium at physiological temperature or under conditions that elicit a cold shock response comprising in the following order: a promoter, at least a portion of a 5'-UTR of a cold shock protein gene, a Shine-Dalgarno sequence, a translational initiation codon, a downstream box of a cold shock inducible gene, and at least one restriction enzyme recognition site for insertion of said heterologous gene.

15
51. The vector of Claim 50, further comprising an additional nucleic acid fragment wherein said fragment encodes a cold shock inducible gene.

52. The vector of Claim 50, further comprising an additional nucleic acid wherein said fragment encodes a heterologous gene.

53. A transformed bacteria containing the vector of Claim 50.

Sub B7
20
54. A method of overexpressing a gene comprising transforming bacteria with a nucleic acid vector of Claim 50 and subjecting said bacteria to conditions that elicit a cold shock response.

55. The method of Claim 54, wherein said conditions that elicit a cold shock response comprise subjecting said bacteria to a sufficiently low temperature to elicit a cold-shock response.

56. The method of Claim 55, wherein said temperature is about 10-15°C.